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APPENDIX A

DRUG report from the Investigational Drugs database

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natalizumab

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Last Updated	17th January 2002
Highest Dev. Status	Phase III clinical
Indication	Inflammatory bowel disease, Multiple sclerosis, Ulcerative colitis, Crohn's disease
Action	Integrin modulator, Cell adhesion molecule inhibitor
Technology	Monoclonal antibody, humanized
Company	Elan Pharmaceuticals Inc.

Summary

Natalizumab, a humanized monoclonal antibody specific for the alpha-4 beta-1 integrin (VLA4) expressed on leukocytes, is being developed by Elan (formerly Athena Neurosciences) and Biogen as a potential treatment for chronic multiple sclerosis (MS), inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC). Several phase II trials have been completed [313095], [275424]. By May 2001, all phase II trials were completed [412878], [413862]. In July 2001, analysts expected that the earliest launch date would be in the second half of 2004 [416802]. Phase III trials for the treatment of MS began in December 2001 [434121]. In January 2002, it was reported that natalizumab had also entered phase III trials for CD [436146].

In December 2001, Elan and Biogen had enrolled and dosed the first patients in their multicenter phase III clinical trials of Antegren (natalizumab) in MS. Elan and Biogen expected to enroll and dose the first patients in their phase III clinical trials for Crohn's disease before the end of 2001 [434121].

The first of the studies conducted was a phase II, double-blind, placebo-controlled trial which involved 213 MS patients at 26 sites in the US, Canada and the UK. Patients (suffering from either relapsing-remitting MS or secondary progressive MS) received monthly doses of natalizumab or placebo over a 6-month period. The primary endpoint, of a reduction in new gadolinium-enhancing lesions compared to placebo over the 6-month treatment period, was achieved with a high degree of statistical significance [396629]. The full phase II study data for multiple sclerosis were presented at the congress of the European Committee for the Treatment and Research in Multiple Sclerosis (ECTRIMS) in September 2001 in Dublin, Ireland. In the placebo group the mean of new gadolinium-enhancing lesions was 9.6, whereas that of the natalizumab-treated groups ranged from 0.6 to 1.2 according to dose group. Treatment was generally well tolerated. Adverse effects included headache, asthenia and urinary tract infections and infrequent hypersensitivity-like reactions. Based on the above data, the companies aim to commence two phase III MS studies, studying natalizumab as a monotherapy as well as in combination with Biogen's interferon beta-1a (qv) [416802], [422339].

In a double-blind, placebo-controlled, phase II study conducted at eight centers in the UK, 70 patients with MS were assessed over a 12-week period. Natalizumab showed a significant reduction in new brain lesions as measured by MRI, compared to placebo [275424]. Further phase II MS results were reported in July 1999. The trial investigated natalizumab in the treatment of acute exacerbation in patients with MS. The results were consistent with results observed in a previous multiple-dose phase II study indicating the potential utility of natalizumab for chronic treatment of patients with MS, but the results do not support further development for the treatment of acute exacerbation; that program has therefore been discontinued [333924].

Crohn's Disease

In May 2001, results of a phase II trial were presented at Digestive Disease Week in Atlanta, GA. A European multicenter study (CD202), sponsored by Elan Pharmaceuticals and Biogen, enrolled 244 individuals with chronic active CD (CDAI score = 220 to 450). Patients received either (i) 6 mg/kg iv natalizumab at weeks 0 and 4; (ii) 3 mg/kg iv natalizumab at weeks 0 and 4; (iii) 3 mg/kg iv natalizumab at week 0 then placebo at week 4; or (iv) placebo at weeks 0 and 4. The primary endpoint was the number of patients in remission at week 6 (CDAI < 150). Many patients were taking concomitant steroids or immunosuppressives, and medication was continued throughout the trial. A significant clinical response (CDAR decrease > 70) to natalizumab was noted at week 2 and was sustained throughout the 12 weeks, with a maximal response of 74% observed in the 3 mg group, compared to 38% in placebo. Remission occurred in 46% of 3 mg group (27% in placebo). In addition, a significant improvement in quality of life, as assessed by questionnaire, was observed. There was no significant difference in adverse events between treatment groups and placebo. There were two incidences of infusion reaction, and one patient expressed antibodies to natalizumab. Further trials were planned with the 3+3 dose regimen at this time [409094], [410251], [412689], [413891]. A pivotal phase III trial was expected in the second half of 2001 [422425].

A separate phase II, double-blind, placebo-controlled study conducted across 38 sites in eight European countries included 240 patients with moderate to severe CD. Patients received doses of natalizumab or placebo at week 0 and week 4. This study also demonstrated statistically significant positive results on multiple endpoints, including induction of remission as measured by the CD activity index. Further information about the potential safety and efficacy of natalizumab were due to be presented at a scientific conference in 2001 [396629].

In two separate pilot studies involving a total of 40 patients suffering from CD or UC, a positive trend towards efficacy was noted and natalizumab treatment was well tolerated in all three studies. Interim analysis of phase II data show positive results [179966], [276967]. Results from a 30-patient CD trial were reported at DDW in May 1999. Natalizumab was shown to be safe and well tolerated at 3 mg/kg. There was a positive efficacy trend [327248]. Serum samples from patients treated with natalizumab showed reduced levels of soluble VCAM-1 and increased

The company conducted UK phase I trials between June and December 1995. These trials involved 35 healthy volunteers, with the aim of determining safety and pharmacokinetic data [179966]. The results showed that the product was well-tolerated and had an acceptable safety and pharmacokinetic profile [200972].

VLA4 specifically binds to VCAM-1, a ligand present on brain endothelial cells, which is a potential mediator of autoimmune disorders, leading to MS [222518].

The humanized antibody, natalizumab, is claimed in the associated patent, WO-09519790. VLA-4 itself and monoclonal antibodies for the integrin were first described in a patent (EP-00330506) by the Dana-Farber Cancer Institute.

In April 2000, Merrill Lynch forecast that the drug would be launched in 2001 [364935]. In September 2000, Merrill Lynch expected NDA filing to take place in 2003, with a launch in 2004 if the on-going development program remains on track [382577]. In August 2000, Merrill Lynch predicted sales of natalizumab of \$50 million in 2002, rising to \$150 million in 2004 [383230]. In September 2000, analysts Merrill Lynch predicted a launch in 2004 with sales estimated at \$50 million for the full year [383742]. In April 2001, ABN AMRO predicted launch in 2004 for MS and Crohn's disease with sales of \$50 million [422425]. In September 2001, Salomon Smith Barney estimated sales of \$20 million in 2004 [422373].

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Name:	Type:
natalizumab	INN
AN-100226	Research Code
alpha4 beta1 integrin MAb / Antegren	Trade Name
MAN100226	Research Code
VLA-4 MAb / Antegren	
CAS	
189261-10-7	Immunoglobulin G ₁ anti (human integrin alpha 4) (human-mouse monoclonal AN100226 gamma 4-chain) disulfide with human-mouse monoclonal AN100226 light chain dimer

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Indication

Antegren is a humanized monoclonal antibody (mAb) against the integrin subunit alpha4 (CD49d). The integrin alpha4 can pair with either of two subunits to form a cell surface heterodimeric receptor, namely, alpha4/beta1 (very late antigen (VLA)-4, CD49d/CD29), or alpha4/beta7. VLA-4 is being vigorously pursued as a therapeutic target for chronic inflammatory diseases due to its relatively well known biology [265753], [265775], [279211]. Currently, Antegren, the most advanced agent in the clinic, is undergoing evaluation as a therapy for MS and inflammatory bowel diseases.

VLA-4 is an active participant in the inflammatory cascade [279211], by virtue of its molecular interactions with alternatively spliced fibronectin-containing CS-1 (CS-1-fibronectin) [279215], [279211], [285283], and vascular cell adhesion molecule (VCAM)-1 [279228], [265775], [279233]. Expression of both CS-1-fibronectin and VCAM-1 has been demonstrated in rheumatoid arthritis [266400], [279238]. In addition, various immunohistopathological studies have shown the presence of either VCAM-1, or CS-1-fibronectin in chronic inflammatory lesions, including atherosclerosis [279240], [279245], arteriopathy [265791], cardiac and kidney allografts [265791], [279249], and glomerulonephritis [279250]. Taken together, these data suggest that VLA-4 mediates selective leukocyte recruitment in chronic inflammatory disorders.

Among human peripheral blood cell populations, alpha4 integrins are expressed on T-lymphocytes and monocytes [265759], [265756], and also on eosinophils [279252], [101722], [279258], [279259], [279261]. In contrast, alpha4 is either absent, or expressed at very low levels, on platelets and neutrophils [265759], [265760], [265763]. Consequently, blockade of alpha4 integrins, eg, VLA-4, is expected to be efficacious in chronic diseases mediated by T-cell, monocyte, or eosinophil infiltration (eg, multiple sclerosis (MS) and asthma). In contrast, VLA-4 therapy is anticipated to have little or no impact in acute inflammation, and cause few general immunosuppressive side effects.

Preclinical Data

A multitude of anti-VLA-4 mAbs have been generated that are specific to mouse, rat and human alpha4 integrin antigens [265753]. In addition, some of these mAbs display immune cross-reactivity with guinea pig, rabbit, sheep, and primate alpha4 [265753]. In summary, the availability of anti-alpha4 mAbs to several animal species has helped elucidate in vivo pathophysiology of VLA-4 in preclinical pharmacology models.

Pharmacology

T-lymphocyte recruitment to the central nervous system (CNS) was one of the first in vivo functions for VLA-4 to be discovered [279263], [265840]. In fact, using mouse encephalitogenic T-cell clones, Janeway and coworkers demonstrated that entry into brain parenchyma by CD4+ T-lymphocytes required high surface expression of alpha4 integrin [265840]. Furthermore, the severity of adoptively transferred experimental autoimmune encephalomyelitis (EAE, a rodent surrogate model of human MS), and the degree of perivascular T-cell infiltration caused by the former clones correlated with alpha4 expression [265840]. Consistent with the role of VLA-4 in EAE, a single administration with either anti-VLA-4, or anti-VCAM-1 at the time of EAE transfer resulted in retardation of clinical symptoms [265840]. This suggests the involvement of the VLA-4/VCAM-1 pathway in mononuclear leukocyte trafficking through the CNS.

The mouse counterpart of Antegren, MAb AN-100226m, has been tested in active, as opposed to passive or adoptively transferred, EAE in guinea pigs [265842]. Not only does treatment with AN-100226m, prior to development of active EAE, suppress the initiation of clinical symptoms, but disease reversal is also observed following AN-100226m administration, after onset of clinical signs [265842]. Moreover, AN-100226m therapy resulted in clearance of monocytes and T-lymphocytes from perivascular and parenchymal areas in the CNS, and demonstrated a neuroprotective effect against demyelination of the white matter [265842]. Similar data were obtained in a separate study from the same investigators using a different anti-alpha4 mAb, administered as late as three and a half weeks after disease onset [279265]. In conclusion, VLA-4 treatment shows efficacy in preclinical disease models of MS, even when therapy commences after neurological symptoms have already become apparent.

Clinical Development

Phase I

A double-blind dose-escalation study of Antegren was performed in patients with clinically diagnosed MS to evaluate

its safety, tolerability, and pharmacokinetic (PK) profile [344444]. Antegren was administered as a single iv infusion at increasing concentrations. Three patients received doses of 0.03, 0.1 and 0.3 mg/kg, respectively while two groups of six patients received 1, and 3 mg/kg. As control, a total of seven additional patients, distributed among the five Antegren dosing groups, received placebo. The drug was safe and well tolerated at all doses tested. Symptoms following treatment were evaluated by recording vital signs, laboratory chemistry, hematology and urinalysis, and electrocardiogram (EKG). Adverse events were generally mild, and no differences were apparent among various dosing groups. Headache was the symptom most frequently reported, but it occurred almost equally in both placebo (86%), and drug (81%) groups. Pharmacokinetic parameters for the 3 mg/kg iv drug infusion showed that Antegren reached a maximum serum concentration (C_{max}) of approximately 50 microg/ml in roughly 2 h (T_{max}), and the half-life was about 4.5 days. Moreover, Antegren could still be detected in circulation 3 to 8 weeks after a single iv dose of 3 mg/kg. In summary, this data justified conducting efficacy studies with Antegren following acute dosing in MS.

Multiple sclerosis

Antegren efficacy was assessed in a double-blind study in patients (n = 72) exhibiting either relapsing-remitting, or secondary chronic progressive MS, who were enrolled at eight sites in the UK [344447]. Each patient received two iv infusions of either Antegren (3 mg/kg), or placebo separated by a 4-week period. The endpoints of the study were lesion activity in MS measured by magnetic resonance imaging (MRI) scans, and overall clinical evaluation, recorded at 12 and 24 weeks after administering the first dose of Antegren. In the first 12 weeks following initiation of treatment, patients in the Antegren group, exhibited fewer new active lesions compared to placebo. In particular, MRI scans showed that the mean cumulative number of new active lesions was 1.8 for the Antegren cohort versus 3.6 (p < 0.05) for placebo. Nevertheless, the total number of exacerbations in the first 12 weeks was not statistically different in the Antegren (n = 9), or placebo group (n = 11). Thus, the data suggest that preservation of blood-brain barrier (BBB) integrity is afforded in the initial 12 weeks after Antegren administration. However, this effect may not be sufficient to have a clinical impact in patients, particularly in terms of MS exacerbations. By week 12, serum titers of Antegren had reached below saturation levels (ie, 1 microg/ml) in the vast majority of treated patients (97%). Consistent with this result, from weeks 12 to 24 after initiation of treatment, the mean cumulative number of new active lesions was not significantly different in the Antegren versus placebo group. Again, this suggests that continuous blockade of alpha4 integrin on lymphocytes is necessary to prevent their entry into the MS brain tissue.

Interestingly, the total number of disease exacerbations was significantly higher in patients treated with Antegren (n = 14) compared to placebo (n = 3, p < 0.05), from week 12 to 24 after initiation of treatment. Thus, a 'rebound' effect may be seen in patients receiving Antegren, after the antibody is cleared from circulation.

Crohn's disease

In two separate pilot studies, Antegren showed a positive trend towards efficacy in the treatment of patients suffering from Crohn's disease or ulcerative colitis (n = 40) [275424]. As with the MS trial, Antegren therapy was well tolerated by these patients [275424].

In the phase I trial, three out of six patients in the highest single dose group, ie, 3 mg/kg, developed anti-Antegren or anti-idiotypic antibodies. However, in the phase II study, only a small proportion of patients in the Antegren group (4/37, 11%) generated antibodies to the drug, even though a 3 mg/kg dose was administered twice. In conclusion, while raising anti-drug antibodies is a concern, phase II trial results indicate that it is a relatively infrequent event. Nevertheless, the potential effect of anti-Antegren on MRI scans, or clinical outcomes for the affected patient population was not investigated further.

In the phase II study, patients treated with Antegren experienced lymphocytosis relative to placebo, even though no significant differences in incidence of other adverse events was noted between the two treatment groups. Between weeks 1 and 12 after initial dosing, there was an increase of 56 to 60% in blood lymphocyte counts in the Antegren cohort, relative to placebo. Lymphocyte numbers remained elevated by week 16, but returned to baseline levels, ie, before initiation of Antegren dosing, by weeks 20, and 24. Taken together, these observations underscore the mechanism of action of Antegren, namely, inhibition of lymphocyte migration. On the negative side, however, they also point to a potentially troubling adverse event, ie, lymphocytosis.

MS is a chronic neurodegenerative disease with autoimmune characteristics, and multi-factorial causes. While the precise agent(s) that triggers disease onset is unknown, T-lymphocyte infiltration into the brain and spinal cord tissue is a well-accepted cardinal sign of MS. Recently, autoantibodies to proteins in the myelin sheath have also been identified in plaque areas in the CNS of MS patients [344449]. Therefore, it appears that both cellular (ie, T-cell), and humoral (ie, antibody) immunity are involved in this demyelinating disease.

Since Antegren is a blocking monoclonal antibody to the alpha4 integrin, one would expect this treatment to prevent T-cell entry into the CNS of MS patients. In fact, this is exactly what the data from a phase II trial of the drug suggests (see above). Moreover, MRI scans of patients treated with Antegren show that fewer new lesions develop in their brains, relative to placebo. While this is potentially a promising result in the long-term, no apparent impact is observed on the number of disease exacerbations in the short-term. Thus, a clear clinical benefit after Antegren therapy still remains to be demonstrated.

A major concern associated with Antegren treatment is its potential effect on normal lymphocyte recirculation through lymph nodes. In addition, the ability of T-cells to mount cellular immune responses to foreign antigens may be affected by the drug, even though no increase in infections was noted in the phase II study. However, the former caveat appears to be justified by the data from the phase II trial, ie, patients receiving Antegren experience a transient lymphocytosis. This observation suggests that the drug may temporarily inhibit T-cell migration into lymphoid tissues. Consequently, the frequency of Antegren therapy may be limited in order to allow a patient's

immune system to restore normal blood homeostasis.

A second concern is the apparent increase in acute episodes after the drug falls below saturation levels from the systemic circulation (see above). A potential explanation may be related to the observed lymphocytosis. This could be consistent with data from the phase II study, since rates of new lesion formation were comparable for the placebo and Antegren groups following drug clearance [344447]. A likely interpretation of this result is that both groups exhibited a similarly compromised BBB, when the alpha4 integrin on T-cells was not blocked. Thus, patients receiving Antegren may have more T-cells infiltrating the CNS parenchyma, simply because they have greater numbers in circulation after the drug has been cleared. In turn, this may translate into more clinical exacerbations in the drug group shortly after Antegren therapy is discontinued.

In conclusion, Antegren appears to be a "near hit", as an editorial comment accompanying publication of the phase II trial argues [344451], but several questions still remain to be resolved. In the meantime, the sponsor of the drug, Elan Corporation of Ireland, states that development of Antegren as an acute MS therapy will not be further supported, shifting its focus instead to chronic treatment of patients with MS [344452].

Name	Summary	Reference
Biogen Inc.	In August 2000, Biogen and Elan announced a worldwide collaboration to develop, manufacture and commercialize Antegren. Costs and benefits will be shared. Financial terms were not disclosed.	379455
Protein Design Labs Inc.	In April 1998, Protein Design Labs (PDL) granted a worldwide, nonexclusive license under its antibody humanisation patents to a subsidiary of Elan for Antegren. Under the terms of the agreement, Elan is paying PDL a non-refundable licensing and signing fee of \$1.3 million. PDL and Elan agreed to make milestone and royalty payments for the licensed products.	286198
Avogen Inc.	Co-developing Antegren for MS as part of a focused drug development with Elan Corp (Athens).	223736

Study Type	Effect Studied	Experimental Model	Results	Reference
In vivo	Reversal of pathology associated with experimental allergic encephalomyelitis	Experimental allergic encephalomyelitis in guinea pigs	Reduction in edema in CNS and blood brain barrier permeability by AN-100226	222522
In vivo	Reversal of pathological signs associated with experimental allergic encephalomyelitis	Experimental allergic encephalomyelitis in guinea pigs	Antibodies against alpha4 beta1 integrin induced a rapid clearance of edema from the CNS and reduction in pathological features including demyelination	265842
In vivo	Reversal of pathology associated with experimental allergic encephalomyelitis	Experimental allergic encephalomyelitis in mice	Antibodies against alpha4 beta1 integrin decreased clinical and histopathological signs, including a reduction of T cell infiltration and a decreased induction of inflammatory cytokines in the brain vessels	265845
In vitro	Lymphocyte and monocyte binding to experimental allergic encephalomyelitis brain vessels	Adhesion assay on tissue sections	Antibodies to alpha4 beta1 integrin inhibited adhesion of lymphocytes and monocytes to brain vessels from experimental allergic encephalomyelitis animals	279263

Study Type	Result	Reference
Development of antibody	Humanization of mouse monoclonal antibody against alpha4 beta1 integrin AN-100226	269705

Study Type	Effect Studied	Experimental Model	Results	Reference
In vivo	Antegren effects on new brain lesion formation in patients with multiple sclerosis	Phase II double-blind placebo-controlled multicentre study in patients	Antegren on day 0 and day 28 of a 22-week study reduced the number of new brain lesions in patients with multiple sclerosis when compared to placebo	275424
In vivo	Effects of Antegren in patients with Crohn's disease or ulcerative colitis	Phase II clinical study	Antegren showed improvement in patients given Antegren	275424